Mycol Progress (2014) 13:1191–1197
DOI 10.1007/s11557-014-1008-9

ORIGINAL ARTICLE

Amanita heishidingensis, a new species of *Amanita* sect. *Lepidella* from China

Fang Li · Qing Cai

Received: 16 June 2014 / Revised: 19 August 2014 / Accepted: 20 August 2014 / Published online: 9 September 2014

Abstract A new species of *Amanita* sect. *Lepidella*, *A. heishidingensis*, is described based on both morphological and molecular evidences. It was compared with similar species and illustrated with line drawings and photographs. This species was found in Heishiding National Nature Reserve, Guangdong Province, South China.

Keywords Amanitaceae · Morphology · Phylogenetic analyses · Taxonomy

Introduction

The genus *Amanita* Pers. in China has been studied extensively in the last twenty years or so and over eighty species including several lethal ones caused many troubles and losses have been reported from China (Yang 1994, 1997, 2005; Zhang et al. 2010; Chen et al. 2014; Deng et al. 2014). Since November 2008, the first author has collected mushrooms in Heishiding National Nature Reserve (111°49′09″–111°55′01″E, 23°25′15″–23°30′02″N), Guangdong Province, China, and found more than 30 taxa of *Amanita* there. One of them, found in forests dominated by fagaceous trees, is described and illustrated herein as a new species.

F. Li (✉)
School of Life Sciences, Sun Yat-sen University,
Guangzhou 510275, China
e-mail: lsslif@mail.sysu.edu.cn

Q. Cai
Key Laboratory for Plant Diversity and Biogeography of East Asia,
Kunming Institute of Botany, Chinese Academy of Sciences,
Kunming 650201, China

Materials and methods

Morphology

The description of the new species is based on morphological studies of fresh material and exsiccata. The photographs (Fig. 1) depict the holotype. Color code follows Kornerup and Wanscher (1978). Tissues were mounted in 5 % KOH and 1 % Congo red for microscopic examination and making of line drawings. Spores were mounted in Melzer's reagent to test for amyloidity. The abbreviation (n/m/p) means n basidiospores measured from m basidiomata of p collections. Dimensions for basidiospores are given using a range notation of the form (a) b-c (d). The range b-c contains a minimum of 90 % of the measured values. Extreme values (a and d) are given in parentheses. Q = length/width ratio of a basidiospore in side view; **Q** = average Q of all basidiospores measured ± sample standard deviation. The holotype collection of the new species was deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Kunming, China. Additional Collections were deposited in HKAS or in the Tottori Mycological Institute (TMI), Tottori, Japan.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from fruiting bodies dried in silica gel according the modified CTAB protocol (Doyle and Doyle 1987). The large nuclear ribosomal RNA subunit of the nuclear ribosomal RNA (nrLSU) was amplified with primers LROR and LR5 (Vilgalys and Hester 1990). The PCR amplification followed those in Zeng et al. (2013) and references therein. The PCR products were then purified using a Gel Extraction and PCR Purification Combo Kit (Spin-colum) (Bioteke, Beijing, China). Sequencing was performed with

Fig. 1 Basidiomata of *Amanita heishidingensis* (Holotype). **a**. Young basidiomata. **b**. Mature basidiomata. **c**. Mature basidiomata, with yellowish white scars on the pileus surface left by detersile volval warts. **d**. Basidiomata including those in a, b, and c. Scale bars: a=2 cm, b, c, d=5 cm

an ABI 3730 DNA analyzer (Applied Biosystem, Foster City, CA, USA) using the same primer pairs used for the PCR.

Sequence alignment and phylogenetic analyses

Four nrLSU sequences of the new species and one nrLSU sequences of *Amanita japonica* extracted in this study were compared with 26 nrLSU sequences retrieved from GenBank (NCBI; http://blast.ncbi.nlm.nih.gov/). These sequences were aligned with MUSCLE v3.8.31 (Edgar 2004), and then manually optimised on BioEdit v7.0.5 (Hall 1999). Gaps were treated as missing data. The maximum likelihood analysis (ML) conducted on RAxML v7.2.6 (Stamatakis 2006) and Bayesian inference (BI) executed on MrBayes V3.2 (Ronquist and Huelsenbeck 2003) were implemented for the phylogenetic analyses. The optimal substitution model for ML and BI analyses was determined using the Akaike Information Criterion (AIC) as implemented in MrModeltest v2.3 (Nylander 2004). The statistical branch support values were evaluated using rapid non-parametric bootstrapping with 1000 replicates in RAxML and using posterior probabilities from BI analysis. The MrBayes analysis was automatically terminated using the stoprul and stopval commands when the standard deviation of the split frequencies fell below 0.01. Chains convergence was further verified using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to confirm sufficiently large ESS values (>200). Subsequently, the sampled trees were summarized after omitting the first 25 % of trees as burn-in using the 'sump' and 'sumt' command implemented in MrBayes.

Results

Morphological analyses

Three collections with over thirty basidiomata of the new species were morphologically examined. For comparison, four collections of *Amanita japonica*, collected from Japan were examined. Our data indicated that the new species is morphologically different from *A. japonica* (see discussion below).

Phylogenetic analyses

A total of 34 nrLSU sequences were used in the phylogenetic analysis (Table 1). The phylogenetic tree inferred from the ML analysis was consistent with that obtained from the Bayesian

Table 1 Voucher information and GenBank accession of *Amanita* included in the molecular phylogenetic analyses

Species	Voucher	Locality	GenBank accession numbers	
			nrLSU	ITS
A. atkinsonia	RET 301–1	Connecticut, USA	HQ539670	–
A. abrupta	BW_HP_101	Massachusetts USA	HQ539660	–
A. conicobulb	PSC 1368	South Australia, Australia	HQ539683	–
A. cokeri	BW_STF 090506–19	Massachusetts USA	HQ539682	–
A. effusa	PSC 2007	South Australia, Australia	HQ539689	–
A. eriophora	RET 350–4	Angkor, Cambodia	HQ539672	–
A. farinacea	PSC 2529	South Australia, Australia	HQ539692	–
A. heishidingensis	HKAS 76122 (holotype)	Guangdong, China	KC429045	KC429051
A. heishidingensis	HKAS 81481	Guangdong, China	KJ922991	KJ922998
A. heishidingensis	HKAS 81484	Guangdong, China	KJ922993	KJ922999
A. heishidingensis	HKAS 82280	Guangdong, China	–	KJ922995
A. heishidingensis	HKAS 82281	Guangdong, China	–	KJ922996
A. heishidingensis	HKAS 82282	Guangdong, China	KJ922992	KJ922997
A. japonica	TMI 26147 (duplicate HKAS 82328)	Tottori, Japan	KJ922990	KJ922994
A. kotohiraensis	MHHNU 7112	Hunan, China	FJ011682	–
A. longipes	RET 360–1	New Jersey, USA	HQ539704	–
A. macrocarp	31939 L	Guangdong, China	KC408378	–
A. manginiana	HKAS 26146	Yunnan, China	AF024463	–
A. manginiana	HKAS 56933	Yunnan, China	KJ466438	–
A. modesta	HKAS 75405	Guangdong, China	KJ466439	–
A. modesta	HKAS 79688	Guangdong, China	KJ466440	–
A. onusta	RET 297–3	New Jersey, USA	HQ539718	–
A. oberwinklerana	MHHNU 7113	Hunan, China	FJ011683	–
A. oberwinklerana	HKAS 77330	Hainan, China	KJ466441	–
A. ochrophylla	PSC 1127	South Australia, Australia	HQ539717	–
A. polypyramis	RET 159–8	Maryland, USA	HQ539723	–
A. pseudoporphyria	HKAS 26143	Yunnan, China	AF024471	–
A. pseudoporphyria	HKAS 56984	Yunnan, China	KC429047	–
A. rhoadsii	DD97/13	North Carolina, USA	AF097391	–
A. rhopalopus	BW_RET 386–3	West Virginia, USA	HQ539733	–
A. smithiana	RET 382–6	California, USA	HQ539740	–
A. solitariformis	DD 97/12	North Carolina, USA	AF097390	–
A. sublutea	PSC 2401	South Australia, Australia	HQ539749	–
A. vestita	HKAS 77277	Hainan, China	KC429044	–
A. virgineoides	HKAS 18394	Sichuan, China	AF024484	–
A. virgineoides	HKAS 77278	Hainan, China	KC429043	–

*Sequences produced in this study in bold

inference, and thus only the ML tree was shown in Fig. 3. Our molecular phylogenetic analysis robustly supported that the new taxon was a separate species and was related to *A. japonica* with moderately statistical support.

Taxonomy

Amania heishidingensis Fang Li & Qing Cai, sp. nov. (Figs. 1–2).

MycoBank: MB 802273.

Etymology: named for holotype locality.

Holotypus: China, Guangdong Province, Fengkai County, Heishiding National Nature Reserve, at 111°49′09″–111°55′01″E, 23°25′15″–23°30′02″N, alt. 190 m, 29 Feb 2012, Fang Li 33 (HKAS 76122); nLSU and ITS sequences generated from the holotype are KC429045 and KC429051, respectively.

Basidiomata (Fig. 1) medium-sized to large. Pileus (4.5) 7–15.5 cm in diam., globose at first, hemispherical when expanding, later convex to plano-convex to flat with slightly depressed centre, with appendiculate smooth margin, white (2A1) when young, then dirty white (2A2-3A2), dingy cream to pale silvery grey or pale brownish grey (a little lighter than 5C3) especially near centre with age, subfelted when young and becoming smooth later, viscid, usually decorated with big, up to 6 mm wide and 6 mm high, grey to brownish grey (5B2, 5C3, 5D3), acute-pyramidal, truncate-pyramidal to verrucose, subfibrillose to felted adnate, subdetersile to detersile volval remnant warts, towards margin these sometimes passing into small, scattered flocks, flat fibrillose scales with somewhat raised amorphous tips or ridges; sometimes warts washed away by rains, leaving yellowish white (4A2–3) prints or scars on pileus (Fig. 1-c); context white, firm, unchanging. Lamellae free, cream (3A2–4A2), rather crowded to moderately crowded, rather broad, up to 18 mm wide, subventricose

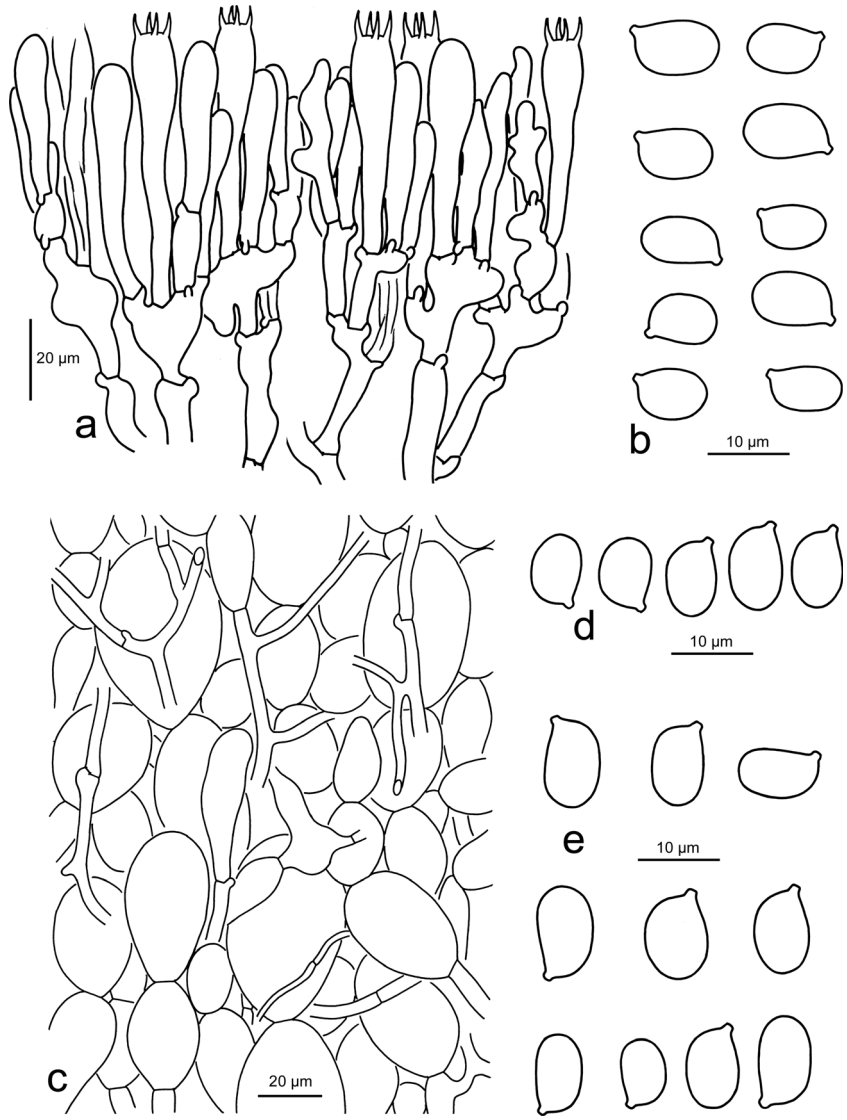

Fig. 2 Microscopic characters of *Amanita heishidingensis* (a-c) and *A. japonica* (d-e). **a**. Hymenium and subhymenium (HKAS 81458). **b**. Basidiospores (HKAS 81458). **c**. Volval remnant on pileus in longitudinal section ((HKAS 81458). **d**. Basidiospores (TMI 1323). **e**. Basidiospores (TMI 26147)

to ventricose, often with smooth edge, occasionally with slightly decurrent edge. Lamellulae attenuate to rounded-truncate, broad, plentiful, in 2 to 3 ranks. Stipe (6) 8–20.5×(0.6) 1.1–2.5 cm, subcylindric or slightly attenuate upwards, with apex slightly expanded, surface white to cream (2A1–3A2), with silk lustre, upper part often covered with white (2A1–2) floccose to farinose squamules, lower part often covered with pale yellow to pale brownish grey (3A2–3, 5B2) floccose recurving squamules; context white to pale cream, solid to fistulose; bulb (1.7) 3–7×(1.5) 2–4.7 cm, napiform, subclavate to ventricose, round based, covered with pale yellow, pale yellowish grey to brownish grey (3A2, 3B2–3, 5B2-5C2) subfelted to subtomentose volval remnants, often exhibiting some longitudinal splitting, with recurving scales often arranged in circles on its upper surface and several to many white mycelical threads on its very base. Annulus apical, felted membranous, friable, with upper surface cream-colored (3A2) and striate; with lower surface whitish, subtomenose to farinose. Spore print white to cream. Odour indistinct.

Lamellar trama bilateral, divergent; mediostratum 30–50 μm wide, filamentous hyphae 2–6 (10) μm wide, branching; vascular hyphae rare; with lateral stratum made up of intercalary inflated elements (25–45×5–20 μm), connected with subhymenium. Subhymenium ca. 20–30 μm thick, with 2–4 layers of subglobose to subcylindric ramose cells (7–18×4–10 μm). Basidia 43–56×(8) 10–13 μm, clavate, 4-spored, occasionally 2-spored; sterigmata 4–6 μm long; basal septa clamped. Basidiospores (Fig. 2-b) [200/9/3] (7) 8–12 (15)×(5) 6–8 (9.5) μm, Q=(1.11) 1.31–1.67 (1.83), **Q**=1.46±0.14, mostly ellipsoid, occasionally broadly ellipsoid or elongate, thin-walled, colorless, smooth, amyloid; apiculus ca. 1 μm long. Lamellar edge sterile, mainly consisting of subglobose to clavate cells (12) 16–25 (40)×5–11 (14) μm, terminal and single or in chains of 2–3 with subterminal cells smaller than terminal ones. Pileipellis (60) 100–140 (200) μm thick; upper layer (10–50 μm thick) strongly gelatinized, composed of subradially interwoven, thin-walled, colorless to subcolorless filamentous hyphae 2–8 μm wide; lower layer (80–125 μm thick) slightly gelatinized to gelatinized, composed of radially and compactly arranged, thin-walled, subcolorless or pale yellowish vacuolar pigmented filamentous hyphae 2–8 (12) μm wide; septa sometimes with clamps; vascular hyphae occasional, branching, sinuous, yellow, 3–8 (16) μm wide. Volval remnants on pileus, upper portion of warts (Fig. 2-c) made up of subvertically arranged elements: inflated cells very abundant to dominant, subglobose, 14–75×14–68 μm, ovoid, to ellipsoid, clavate or sphaeropedunculate, (18) 25–135×(10) 20–70 μm, becoming larger towards the pileipellis, terminal singly or in chains of 2–3 with wall ca. 0.5 μm thick and hyaline, often with brownish to yellowish vacuolar pigments, occasionally colorless; filamentous hyphae fairly abundant, 1.5–8 μm wide, frequently branching, with walls thin and hyaline, colorless or with brownish to yellowish vacuolar pigments; septa sometimes with clamps; vascular hyphae rare to locally conspicuous; base of warts 100–160 μm thick, filamentous hyphae somewhat more abundant than in other parts of warts, and gelatinized. Volval remnants on upper part of bulb composed of inflated cells and filamentous hyphae, inflated cells subglobose, ovoid, ellipsoid, pyriform or sphaeropedunculate, 18–110×18–36 μm, abundant, periclinally arranged near bulb surface, becoming smaller and irregularly arranged outwards; filamentous hyphae 2–8 μm wide, abundant; vascular hyphae 5–15 μm wide, conspicuous, branching. Stipe trama longitudinally acrophysalidic; filamentous hyphae 4–10 μm wide, abundant, septa often clamped; acrophysalides 140–230×20–25 μm, dominant; vascular hyphae 8–33 μm wide, locally conspicuous to abundant. Annulus composed of 2–9 μm wide, colorless, hyaline, fairly abundant to abundant filamentous hyphae, with septa often clamped; inflated cells very abundant, sphaeropedunculate, pyriform to subglobose, 20–50×17–45 μm, usually single, colorless and with walls thin and hyaline; vascular hyphae locally conspicuous, sinuous, 3–10 μm wide.

Habitat and distribution: gregarious or scattered on soil in forests dominated by *Fagaceae*, at 190–600 m alt. Presently known only from Heishiding National Nature Reserve, Guangdong Province, China.

Additional specimens examined: China, Guangdong Province, Heishiding National Nature Reserve, alt. 190 m, 18 February 2014, Fang Li 1580 (HKAS 81458–81480, HKAS 82279–82283); the same place, alt. 600 m, 5 March 2014, Fang Li 1581 (HKAS 81481–81484, HKAS 82284–82292).

Specimens of Amanita japonica *examined*: Japan, Tottori Prefecture, Katsurami, 01 October 2007, Yukihiro Nishio TMI 26147 (duplicate HKAS 82328); same location, 26 July 2011, Yukihiro Nishio TMI 26146 (duplicate HKAS 82329); Japan, Shiga Prefeture, Otsu City, Ishiyama-Terabe, 17 August 1973, Z. Sugiyama & E. Nagasawa TMI 1322 (duplicate HKAS 82330); Japan, Shiga Prefeture, Otsu City, Ishiyama-senjo, 14 Sepetember 1973, E. Nagasawa TMI 1323 (duplicate HKAS 82331).

Discussion

Amanita heishidingensis, a member of *Amanita* sect. *Lepidella* (Bas 1969), is characterized by its medium-sized to large basidioma with a dirty white to whitish viscid pileus covered with grey to brownish grey pyramidal to verrucose volval remnants, light cream lamellae, a whitish stipe covered with white to pale brownish grey floccose to farinose recurving squamules, usually with a big napiform, subclavate to ventricose bulb covered with pale yellow to pale brownish

grey subfelted to subtomentose volval remnants, a fugacious annulus, ellipsoid amyloid spores, and abundant clamps in all tissues.

Amanita heishidingensis keyed out in *Amanita* subsect. *Solitaria* Bas stirps *Solitaria* (Bas 1969). It resembles *A. japonica* Hongo ex Bas. *Amanita japonica*, originally described from Japan (Bas 1969; Imazeki and Hongo 1987), resembles *A. heishidingensis* by its similarly shaped basidioma, the greyish voval warts on the cap, and the similar basidiospores. But the main morphologial and anatomical differences in basidioma can distinguish *A. japonica* from *A. heishidingensis*. On the morphologial features, *A. japonica* generally has a medium-sized basidioma (cap 55–80 mm wide, stipe 80–170× 7–15 mm); a small fusiform-rooting to subclavate bulb (up to 25 mm wide); a dry, moderately dark grey to pale buffy grey, felted-subflocculose pileus; a non-gelatinized pileipellis; small (about 2 mm wide and 1.5 mm high) subpyramidal warts adnate on the mature pileus; subflocculose edged lamellae (Bas 1969). While *A. heishidingensis* usually has a big sized basidioma (cap 70–155 mm wide, stipe 80–205×11–25 mm) with a big napiform to subclavate bulb (20–47 mm wide), with the base always round, not rooting; a viscid, white to whitish (without greyish tint when young), glabrous pileus with the pileipellis always strongly gelatinized; the warts on the pileus are much larger (up to 6 mm in wide and high); the pileipellis under the warts is often strongly gelatinized-making the warts detersile and easily washed off in rainy weather; lamellar edge is always smooth. On the anatomical features, the pileipellis of *A. japonica* is difficult to delimited from the greyish volval remnants over it, not or very slightly gelatinized, while *A. heishidingensis* has a clear delimited, gelatinized to strongly gelatinized pileipellis; hyphae and inflated cells in the warts of *A. japonica* are much more darker colored than those in *A. heishidingensis*; the annulus of *A. japonica* has abundant to nearly abundant hyphae, with inflated cells mostly clavate shaped, and with yellowish vacuolar pigmentation in both hyphae and inflated cells, while *A. heishidingensis* has fewer hyphae in its annulus, with inflated cells mostly globose, subglobose to sphaeropedunculate, and has rare vacuolar

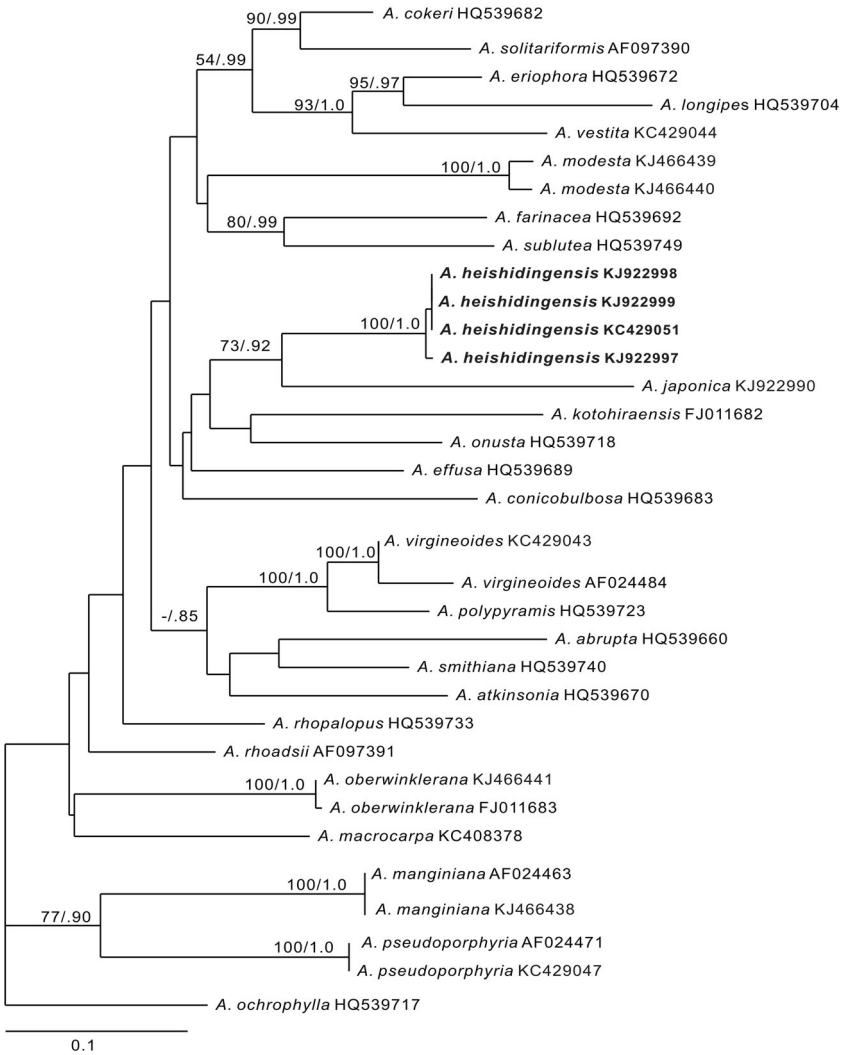

Fig. 3 Phylogenetic tree inferred from maximum likelihood (ML) analysis based on nrLSU sequences of *Amanita* sect. *Lepidella*. ML bootstrap over 50 % and Bayesian posterior probabilities over 0.80 were reported

pigments in both hyphae and inflated cells. Furthermore, *A. heishidingensis* grows in the early spring, when the temperature is average 5–12 °C, never over 20 °C, and can't be found in late spring, summer or autumn; while *A. japonica* grows in late summer to early autumn, when the weather is much warmer. Our molecular phylogenetic analysis also suggested that *A. heishidingensis* and *A. japonica* are different species (Fig. 3).

Amanita cokeri (E.J. Gilb. & Kühner) E. J. Gilb., originally described from North America (Bas 1969), resembles *A. heishidingensis* in similar shape of basidioma and white pileus covered with rather larger white to brownish pyramidal warts. But *A. cokeri* can easily be differentiated from *A. heishidingensis* by its distinctive ample double ring, and somewhat larger basidiospores (11–13.5×7–9 μm) (Bas 1969). Additionally, warts of *A. heishidingensis* are never white at any stage of development, and always having a greyish tint; moreover the bases of warts are not radially fibrillose; and the gills are never pinkish at any stage of development. The molecular phylogenetic analysis also indicated that the two species are distinct.

Amanita miculifera Bas & Hatanaka originally described from Japan (Bas and Hatanaka 1984), resembles *A. heishidingensis* in its similar sized and shaped basidioma, whitish-greyish (between 1A1 and 1B1) pileus covered with moderately grey subpyramidal warts, and similar sized and shaped basidiospores; but its pileus is conical with obtuse apex to plano-conical; its lamellae are rather narrow; its bulb is strongly rooting; its warts are much smaller and the arrangement of the inflated cells in its volval warts is quite irregular (Bas and Hatanaka 1984).

Acknowledgments The authors are grateful to Dr. Rodham E. Tulloss for his critically reviewing the manuscript. They are also grateful to Mr. Eiji Nagasawa for his critically reviewing the manuscript, and for his precious gift of *Amanita japonica* specimens. Thanks are also due to Dr. Zhu L. Yang for his encouragement and for providing the line-drawings. This study is financed by School of Life Sciences, Sun Yat-sen University.

References

Bas C (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5:285–597

Bas C, Hatanaka S-I (1984) An undescribed species of *Amanita* section *Lepidella* from Japan. Persoonia 12:321–325

Chen ZH, Zhang P, Zhang ZG (2014) Investigation and analysis of 102 mushroom poisoning cases in Southern China from 1994 to 2012. Fungal Diversity 64:123–131

Deng WQ, Li TH, Li P, Yang ZL (2014) A new species of *Amanita* section *Lepidella* from South China. Mycological Progress 13:211–217. doi:10.1007/s11557–013–0906–6

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bull 19:11–15

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. doi:10.1093/nar/gkh340

Imazeki R, Hongo T (1987) Colored Illustrations of Mushrooms of Japan Vol. 1. Hoikusha, Tokyo, pp 115–135

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Kornerup A, Wanscher JH (1978) Methuen handbook of colour. Eyre Methuen, London, p 252

Nylander JAA. 2004. MrModeltest v2.2 Program distributed by the author. In: Evolutionary Biology Centre, Uppsala University.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12):1572–1574. doi:10.1093/bioinformatics/btg180

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21):2688–2690. doi:10.1093/bioinformatics/btl446

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172(8):4238–4246

Yang ZL (1994) Studies of the genus *Amanita* from southwestern China (I). Mycotaxon 51:459–470

Yang ZL (1997) Die *Amanita*-Arten von Südwestchina. Bibl Mycol 170: 1–240

Yang ZL (2005) Flora fungorum sinicorum, vol 27 Amanitaceae (in Chinese). Science Press, Beijing

Zeng NK, Tang LP, Li YC, Tolgor B, Zhu XT, Zhao Q, Yang ZL (2013) The genus *Phylloporus* (Boletaceae, Boletales) from China: morphological and multilocus DNA sequence analyses. Fungal Diversity 58:73–101. doi:10.1007/s13225–012–0184–7

Zhang P, Chen ZH, Xiao B, Bao HY, Tolgor B, Yang ZL (2010) Lethal amanitas of East Asia characterized by morphological and molecular data. Fungal Diversity 42:119–133. doi:10.1007/s1322–010–0018–4